Pakistan Journal of Marine Sciences, Vol.2(2), 113-117, 1993

COMPARATIVE HAEMAGGLUTINIC ACTIVITY IN THE SPECIES OF *CAULERPA* AND *ULVA* (CHLOROPHYTA) OF KARACHI COAST

M.T. Amjad and Mustafa Shameel
Department of Botany, University of Karachi, Karachi-75270, Pakistan

ABSTRACT: Aquous extracts of 24 species of green seaweeds, 13 of *Caulerpa* Lamour and 11 of *Ulva* Linn., were collected from the coastal areas near Karachi, Pakistan and tested for haemagglutination against human erythrocytes of blood groups A,B, AB and O and compared. All the species of *Caulerpa* and seven of *Ulva* exhibited a positive activity, the former genus (a member of Bryopsidophyceae) appeared to possess more phycohaemagglutinins than the latter (a member of Ulvophyceae). The haemagglutinic activity proved to be quite helpful in the distinction of various species of both the genera.

KEY WORDS: Phycohaemagglutinins - lectins - *Caulerpa* spp. - *Ulva* spp. - Chlorophyta - Karachi coast.

INTRODUCTION

The haemagglutinic activity of plants was known since the last century, when it was observed that an extract of *Ricinus communis* L. agglutinated erythrocytes of different mammals. The immunological, nutritional, chemical and other aspects of phycohaemagglutinins and lectins of higher plants have been thoroughly investigated (Lis and Sharon, 1973; Bird, 1977; Jeffe, 1977, 1983; Oppenheimer, 1977; Sharon and Lis, 1977; Cheeke and Shull, 1985), but there are only a few reports on the preliminary screening of haemagglutinic activity of seaweeds (Boyd *et al.*, 1966; Blunden *et al.*, 1975; Fabregas *et al.*, 1984, 1985, 1986; Naqvi *et al.*, 1992). In this communication an attempt has been made to compare the haemagglutinic activity of various species of *Caulerpa* Lamour. and *Ulva* Linn., growing in the coastal waters of Karachi, with the intention to obtain some information about specific differentiations.

MATERIALS AND METHODS

The seaweeds were collected from the rocky ledges and sandy bays near Goth Haji Ali (French Beach), Buleji, Karachi, Pakistan during September 1991 to May 1992. The healthy specimens of each species were selected, thoroughly washed to remove epiphytes, epizoons and attached debris, dried in shade and minced. The chopped and ground material was soaked in distilled water in a percolater for 1 month under sterile conditions. It was then filtered and the extracts were concentrated under reduced pressure below 30°C using a thin film rotary evaporator. The concentrated extract was lyophilized in Eyela Freeze Dryer model FD-1 (Tokyo Rikakikai), thereafter completely dried and powdered. About 5 mg of the powdered extract was dissolved in 10 ml of distilled water and serial double dilutions of the extracts (1:2, 1:4, 1:8, 1:16, 1:32 and 1:64) were prepared.

The phosphate buffer (pH 7) was prepared by mixing 35 ml of stock solution of $NaH_2PO_4.2H_2O$ (26 g/l) and 65 ml of stock solution of anhydrous Na_2HPO_4 (18 g/l). Samples of Rh^+ human blood were obtained from Diagnostic Pathological Labs. (Al-Tashkhees), Block C-1, Doctors Plaza, Taj Complex, M.A. Jinnah Road, Karachi, and erythrocytes (RBCs) were collected by simple centrifugation. A 2% RBC suspension was prepared in phosphate buffer. Haemagglutinic activity was investigated serially in six dilutions of each seaweed extract against all the four blood groups (A, B, AB and O). For this purpose 1 ml of each dilution was added to 1 ml of 2% suspension of erythrocytes in a test tube of 5 ml capacity followed by incubation in a water bath at 25°C. Controls were also run similarly in distilled water instead of seaweed extract. A rough granular deposition in the tube due to agglutination of erythrocytes showed a positive activity, while the smooth button formation due to settling of erythrocytes at the bottom indicated a negative reaction.

RESULTS AND DISCUSSION

Twenty-four species of *Caulerpa* and *Ulva*, collected from the coastal areas of Karachi, were tested for their haemagglutinic activity, out of which 20 showed positive results (Table I). No doubt, some of these species exhibited haemagglutination against only one type of blood group or under a few dilutions only, but as a whole 83% of the investigated species of green seaweeds were found to be bioactive. Naqvi *et al.* (1992) observed only 52% positive activity in the species of green seaweeds of Karachi seashore. However, 80% of the brown seaweeds investigated from the north western coast of Spain (Fabrigas *et al.*, 1986) exhibited the haemagglutinic activity.

The eleven investigated species of *Ulva* showed specific variations in their haemagglutinic activity, four of them were completely inactive, three partially active against one or two blood groups and four completely active. Naqvi *et al.* (1992) investigated nine of these species from Karachi coast and observed a negative response to agglutination in all of them under all the dilutions. However, Blunden *et al.* (1975) observed a positive activity in *U. lactuca,* and a similar activity was noticed in other species of *Ulva* by Boyd *et al.* (1966). The different results obtained by Naqvi *et al.* (1992) are probably due to their extraction technique, as they obtained ethanolic extract of seaweeds, during which some of the phycohaemagglutinins were lost, denatured or changed their structure. It is, therefore, advisable to obtain aquous extracts of seaweeds for agglutination studies, as was done by previous workers (Boyd *et al.*, 1966; Blunden *et al.*, 1975). However, no species of *Ulva* showed a positive activity against all the blood groups at the least dilution (1:2).

Ulva anandii exhibited positive agglutination against all the four blood groups, while *U. bifrons* showed a complete negative reaction in all the dilutions. This indicated that they are completely different species. They were previously included under a single specific epithet *i.e. U. indica* (Anand , 1940), but have recently been separated form one another (Amjad and Shameel, 1993). *Ulva reticulata* showed a positive haemagglutinic activity against all the blood groups and *U. saifullahii*

Table I: Haemagglutinic activity (+) of six dilutions of the aquous extracts of different species of *Caulerpa* and *Ulva* against human erythrocytes (1=1:2, 2=1:4, 3=1:8, 4=1:16, 5=1:32 and 6=1:64 dilutions).

Seaweeds species	Blood group A	Blood Group B	Blood group AB	Blood group O
	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6
Caulerpa chemnitzia (Esper) Lamour.	+ + + + + +	+ + + + + +	+ + + + + +	- - - - - -
C. faridii Nizam.	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +
C. fastigiata Mont.	- - + + + +	+ + + + + +	- - + + + +	+ + + + + +
C. lentillifera J. Ag.	+ + + + + +	+ + + + + +	+ + + + + +	- - + + + +
C. manorensis Nizam.	+ + + + + +	+ + + + + +	+ + + + + +	- - - - - -
C. peltata Lamour.	+ + + + + +	- - + + + +	- - + + + +	- - - + + +
C. qureshii Nizam.	- - + + + +	- - - - + +	- - + + + +	- - - - + +
C. racemosa (Forssk.) J. Ag.	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +
C. scalpelliformis (R. Brown) C. Ag.	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +
C. sertularioides (Gmel.) Howe	- + + + + +	- + + + + +	- + + + + +	- + + + + +
C. taxifolia (Vahl) C. Ag.	- + + + + +	- + + + + +	- + + + + +	- + + + + +
C. veravalensis Thivy *et* Chauhan	- - - - - -	- - - - - -	- - - - - -	- - + + + +
C. vickersiae Borg.	+ + + + + +	- - + + + +	- - + + + +	+ + + + + +
Ulva anandii Amjad *et* Shameel	- - + + + +	- - + + + +	- - + + + +	- - + + + +
U. bifrons Ardré	- - - - - -	- - - - - -	- - - - - -	- - - - - -
U. fasciata Delile	- - - - - -	- - - - - -	- - - - - -	- - - - - -
U. fenestrata Post. *et* Rupr.	- - - - - -	- - - - - -	- - - - - -	- - - - + +
U. grandis Saif. *et* Nizam.	- - - - - -	- - - - - -	- - - - - -	- - - - - -
U. lactuca Linn.	- + + + + +	- + + + + +	- + + + + +	- + + + + +
U. reticulata Forssk.	- - + + + +	- - + + + +	- - + + + +	- - + + + +
U. rigida C. Ag.	- - - - - -	- - - + + +	- - - + + +	- - - - - -
U. sorensenii Chapm.	- - - - - -	- - - - + +	- - - - + +	- - - + + +
U. saifullahii Amjad *et* Shameel	- - - - - -	- - - - - -	- - - - - -	- - - - - -
U. taeniata (Setch.) Setch. *et* Gardn.	- + + + + +	- + + + + +	- + + + + +	- + + + + +

Similarly, *U. fasciata* exhibited strong haemagglutinic activity against all the blood groups and *U. fasciata* did not, showing specific differences between the two. Previously, they were considered as conspecific (Saifullah and Nazimuddin, 1977), but the Karachi specimens have recently been separated as distinct species (Amjad and Shameel, 1993) and the current study further supports this separation.

The thirteen investigated species of *Caulerpa* also showed specific variations regarding their haemagglutinic activity (Table I), three of them were completely active in all the dilutions against all the blood groups, seven were partially active in certain dilutions against all the four blood groups, two were active against three blood groups, and one was active against only one blood group. The species of *Caulerpa,* which is a member of the class Bryopsidophyceae, exhibited much more agglutinic activity than those of *Ulva*, which belongs to the class Ulvophyceae. This difference in activity might be due to their different taxonomic positions.

Caulerpa manorensis, C. qureshii and *C. veranalensis* exhibited remarkable differences in their haemagglutinic activity, indicating that they are distinct species. Coppejans and Meinesz (1988) recorded *C. manorensis* from Paupa New Guinea, discussed its similarities with *C. qureshii* and *C. veranalensis,* and considered the last two as conspecific. Recently, Shameel and Shaukat (1992) have made a taxometric study of these species and from hierarchical agglomerative clustering of their operational taxonomic units (OTUs) showed three distinct groups at 85% similarity level, indicating that they are separate species. The fatty acid composition of these species has further revealed that they are independent and widely different species (Shameel and Khan, 1991). Similarly, *C. chemnitzia, C. pelata* and *C. racemosa* also differ in their haemagglutinic activity from one another, indicating that they are distinct species. They were considered as conspecific by some phycologists, e.g. Silva *et al.* (1987).

The haemagglutinins, which cause the clumping of RBCs, are proteinaceous secondary metabolites (Fabregas *et al.*, 1984). They produce adverse effects like reduced growth, diarrhoea, decreased nutrient absorption and increased incidence of bacterial infections in human body (Jeffe, 1977). They bind themselves with the cells in the intestinal wall and cause a nospecific interference with the nutrient absorption (Jeffe, 1983). The haemagglutinins also impair the immune system, thus enhancing the susceptibility for bacterial infections (Cheeke and Shull, 1985). Some of the phytohaemagglutinins have been found useful in blood banking (Bird, 1977), therefore, it appears worthwhile to investigate chemically the phycohaemagglutinins isolated from seaweeds.

REFERENCES

Amjad, M.T. and M. Shameel. 1993. Two new species and two new reports of *Ulva* L. (Ulvophyceae) from the coast of Karachi, Pakistan. *Pakistan Journal of Marine Sciences* 2: 5-17.

Anand, P.L. 1940. *Marine Algae from Karachi. I. Cholorophyceae.* Punjab University Botanical Publication., Lahore, Pakistan. Pp. 1-52.

Bird, G.W.G. 1977. Lectins in blood banking: A brief review. *Biotesting Bulletin* 2: 2-12.

Blunden, G., D.J. Rogers and W.F. Farnham. 1975. Survey of British seaweeds for hemagglutinins. *Lloydia* 38: 162-168.

Boyd, W.C., L.R. Almodovar and L.G. Boyd. 1966. Agglutinins in marine algae for human erythrocytes. *Transfusion* (Philadelphia) 6: 82-85.

Cheeke, P.R. and L.R. Shull. 1985. *Natural Toxicants in Feeds and Poisonous Plants.* Avi. Publication Co. Inc., Connecticut, USA. Pp. 240-244.

Coppejans E. and A. Meinesz. 1988. Marine algae of Paupa New Guinea (Mandang Prov.) I. Caulerpaceae (Chlorophyta-Caulerpales). *Blumea* 33: 181-196.

Fabregas, J.A., A. Munoz, J. Liovo and J. Abalde. 1984. Agglutinins in marine red algae. *IRCS Medical Sciences* 12: 298-299.

Fabregas, J., J. Liovo and A. Munoz. 1985. Hemagglutinins in red seaweeds. *Botanica Marina* 28: 517- 520.

Fabregas, J., A. Munoz and J. Liovo. 1986. Hemagglutinins in brown seaweeds. *Journal of Experimental Biology and Ecology* 97: 213-219.

Jeffe, W.G. 1977. Immunology of plant lectins. In: *Immunological Aspects of Foods* (Ed. N. Catsimpooles). John Wiley & Sons, New York. Pp. 1-170.

Jeffe, W.G. 1983. Nutritional significance of lectins. In: *Handbook of Naturally Occurring Food Toxicants* (Ed. M. Rechcigl Jr.). CRC Press Inn., Boca Raton, Florida. USA Pp. 31-38.

Lis, H. and N. Sharon. 1973. The biochemistry of plant lectins (phytohemagglutinins). *Annual Review of Biochemistry* 42: 541-545.

Naqvi, S.B.S., D. Sheikh, K. Usmanghani, M. Shameel and R. Sheikh. 1992. Screening of marine algae of Karachi for haemagglutinin activity. *Pakistan Journal of Pharmaceutical Sciences* 5: 129-138.

Oppenheimer, S.B. 1977. Interaction of lectins with embryonic cell surface. *Current Topics of Developmental Biology* 11: 3-5.

Saifullah, S.M. and M. Nizamuddin. 1977. Studies of the marine algae from Pakistan: Ulvales. *Botanica Marina* 20: 521-535.

Shameel, M. and R. Khan. 1991. Fatty acid composition of nine green seaweeds. *Botanica Marina* 34: 501- 504.

Shameel, M. and S.S. Shaukat. 1992. A taxometric study of three related species of *Caulerpa* (Bryopsidophyceae) from northern Arabian Sea. *Marine Research* 1: 1-7.

Sharon, N. and H. Lis. 1977. Lectins: Cell agglutiniating and sugar specific proteins. *Science* 177: 949-950.

Silva, P.C., E.G. Menez and R.L. Moe. 1987. Catalog of the benthic marine algae of the Philippines. *Smithsonian Contributions to the Marine Sciences* 27: 1-179.